UNIVERSITI TEKNOLOGI MARA

OVEREXPRESSION OF HOST FURIN PROTEASE AND INHIBITORY ACTIVITIES OF SYNTHETIC CHALCONES- AND AZEPINES- DERIVATIVE COMPOUNDS TOWARD DENGUE VIRUS TYPE-2

KHUZAIDATUL AZIDAH BINTI AHMAD NAZRI

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of the requirements for the degree of
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Faculty of Medicine

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 10th May 2016 to conduct the final examination of Khuzaidatul Azidah Binti Ahmad Nazri on her Master of Science thesis entitled "Overexpression of host furin protease and inhibitory activities of synthetic chalcones- and azepines-derivative compounds toward dengue virus type-2" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

Harbindar Jeet Singh, PhD
Professor
Faculty of Medicine
Universiti Teknologi MARA
(Chairman)

Sarni Mat Junit, PhD
Associate Professor
Faculty of Medicine
Universiti Malaya
(External)

Zaini Mohd Zain, PhD
Associate Professor
Faculty of Applied Science
Universiti Teknologi MARA
(Internal)

DR MOHAMMAD NAWAWI
DATO' HAJI SEROJI
Dean
Institute of Graduate Studies
Universiti Teknologi MARA
Date: 3rd August 2016

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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Name of Student	:	Khuzaidatul Azidah Binti Ahmad Nazri
Student I.D. No.	:	2011196105
Programme	:	Master of Science – MD 780
Faculty	:	Medicine
Thesis	:	Overexpression of Host Furin Protease and Inhibitory Activities of Synthetic Chalcones- and Azepines-Derivative Compounds Toward Dengue Virus Type-2
Signature of Student	:	.....
Date	:	August 2016

ABSTRACT

The outbreak of dengue disease continues to occur despite extensive measures of vector control. Meanwhile, progress in the vaccine development is often affected by various challenges thus delaying in the production of an effective vaccine across all four dengue serotypes. Thus, efforts in combating dengue are being channelled to other alternatives such as dengue antivirals. The search for this therapeutics has given rise to various screening methods to test for potential inhibitory activities of purified as well as synthetic compounds. Therefore, one of the aims of this study is to produce a furin recombinant protein. This current study also aims to determine the inhibitory effect of the synthetic chalcones- and azepines-derivatives toward dengue virus infection *in vitro*. To achieve the first objective, the furin gene isolated from HepG2 cells inoculated with dengue virus type-2 (DENV2) was cloned and overexpressed in the *E.coli*. The protein lysates of the overexpressed protein were purified using Ni^{2+} (resin) affinity chromatography and its concentration was measured by Bradford assay. The purified furin was confirmed by SDS-PAGE and Western blot analysis. The result showed that the furin is expressed at 60 kDa and was positive toward Rabbit Monoclonal Anti-Furin antibody. Subsequently, two groups of synthetic chalcones (2446DA and 20H46DA) and azepines (MA13, MA15 and MA16) derivatives were measured for their inhibitory activities toward dengue infection by cytotoxicity assay, plaque assay, indirect immunostaining, *in vitro* inhibition assay and fluorescence scanning microscopy. The cytotoxicity assay results showed that the concentration below maximum non-toxic dose (MNTD) for both 2446DA and 20H46DA in HepG2 cells was 15 μg/mL. The same value was obtained for MA15 and MA16. However, MA13 was observed to be less toxic compared to all test compounds with MNTD of 30 μg/mL. The plaque forming unit per ml (pfu/ml) was reduced prominently by 10 to 1000 fold when the infected BHK21 cells were treated with the highest non-toxic concentration compared to lowest non-toxic concentration. The indirect immunostaining results showed a similar trend of virus particles reduction on infected HepG2 cells in both the chalcones- and azepines-derivatives when treated at simultaneous- and post- conditions. However, the azepines MA13 exhibited the most potent activity towards DENV2 whereby total inhibition of virus particles was observed during simultaneous-treatment condition. The *in vitro* inhibition assay results showed that at concentration below MNTD, all compounds exhibited inhibitory activity against DENV2 in a dose dependent manner, indicated by the absence of cytopathic effects. The inhibitory potency strength exhibited was between 73% to 100% against both 1000 $TCID_{50}$ ($p>0.05$) and 100 $TCID_{50}$ ($p<0.05$). Results of the fluorescence scanning microscopy showed that all cytoskeletal changes induced by DENV2 infection were managed by the inhibitory activity of chalcones- and azepines-derivatives in BHK21 cells. In conclusion, we proposed that the purified furin host protease to have high substrate specificity and highly potential to be developed as screening tool for anti-furin compounds. More importantly, the synthetic chalcones- and azepines-derivatives are suitable candidates for the development of therapeutics against dengue infection.

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